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Polydisperse Ethoxylated Fatty Alcohol Surfactants as Accelerators of Cuticular Penetration. 1. Effects of Ethoxy Chain Length and the Size of the Penetrants

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Abstract: The effects of polydisperse ethoxylated fatty alcohol (EFA) surfactants on the penetration of six organic compounds varying in size (molar volumes, 107–282 cm^3 mol^{-1}) and lipophilicity (log K_{ow} 0·8–6·5) were investigated using astomatous isolated cuticular membranes (CM) of *Citrus* and pear leaves. Mobilities of model compounds in CM were measured by unilateral desorption from the outer surface (UDOS). Rate constants (k*) obtained in these experiments are directly proportional to diffusion coefficients and, in the absence of EFA, k* values decreased by a factor of 52 when molar volumes increased only 2·64-fold. Under UDOS conditions using micellar surfactant solutions as desorption media, surfactants are sorbed in the CM and the volume fractions sorbed were found to decrease from approximately 0·062 to 0·018 when the average number of ethoxy groups (nE) increased from 5 to 17. In the presence of the EFA surfactants in the CM, solute mobilities increased markedly though this effect diminished with increasing nE. Surfactants with $nE = 17$ affected solute mobilities only marginally. Surfactant effects on solute mobility increased with the size of the solutes leading to almost identical mobilities of the model compounds. With the current range of our model compounds, lipophilicity increased with increasing molar volumes, though evidence is presented showing that the mobilities of solutes depend on their molar volumes while lipophilicity has no effect. Effects of micellar aqueous solutions of polydisperse surfactants on solute mobilities followed the pattern observed with monodisperse ones.

Experiments simulating foliar uptake (SOFU), by applying 5-μl droplets of solute and increasing amounts of surfactant (1–20 g $litre^{-1}$) to the outer surface of the CM and then monitoring the rates of appearance at the inner surfaces of the CM, were carried out with 1-(3-fluoromethylphenyl)-5-phenoxy-5*H*-1,2,3,4-tetrazole (WL110547; log $K_{ow} = 3·6$) and cyanazine (log $K_{ow} = 2·1$) and the surfactants 'Genapol' C-050 (GP C-050, nE 5) and 'Genapol' C-200 (GP C-200, nE 17). In the applied range, uptake increased with increase in amount of both surfactants and both surfactants were more effective than polyethylene glycol (PEG 400). The smaller, more lipophilic GP C-050 was able to increase the rates of uptake of both compounds greatly, while GP C-200 had a less, though observable, effect, similar to the pattern observed in the UDOS experiments. The results indicated that GP C-050 penetrated the cuticle rapidly while sorption of GP C-200 was slower, though better from a concentrated residue in SOFU experi-

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ments than from aqueous solution in UDOS experiments. This induced some acceleration effects by GP C-200 in SOFU experiments, but these could be countered by reductions in concentrations, and hence driving forces, for penetration at higher levels of application.

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1 INTRODUCTION

Pesticides formulations for foliar application generally contain surfactants. With regard to their intended functions surfactants have been classified by McWhorter and Van Valkenburg as (i) spray modifiers which increase spray retention and coverage of the targets and (ii) activators which improve biological effectiveness of active ingredients.[1,2] In more recent work utilising radio-labelled active ingredients, a better understanding of the mode of action of activator adjuvants has been attempted[3–10] but, despite these efforts, the exact sites and mechanisms of action of these activators have remained unclear, largely due to the complexity of the foliar uptake process.[11,12] Rates of foliar uptake are proportional to permeabilities of cuticles and to driving forces and these factors cannot be separated when only amounts or rates of foliar uptake from droplets are measured.[13] Permeabilities of cuticles have been studied using isolated cuticles and the system aqueous donor/cuticle/aqueous receiver. In this type of study, driving forces are known and permeabilities can be calculated from the rates of penetration. Such studies have demonstrated that permeabilities of cuticles can be increased by certain surfactants[7,14–19] and, since the surfactants cannot affect the concentration, and hence the driving force, of the solute, the most likely explanation is that the surfactants have increased the permeability of the cuticles. This was demonstrated directly by using a new experimental procedure called UDOS (unilateral desorption from the outer surface).[17,20,21] With this method, solute mobilities in cuticles and effects of adjuvants on solute mobility in cuticles can be measured and diffusion coefficients estimated.[22] Studies with monodisperse ethoxylated alcohols applied as aqueous micellar solutions revealed that (i) alcohols were most effective, (ii) polyethylene glycols had no influence on solute mobility in cuticles, (iii) ethoxylation decreased effects on solute mobility, (iv) effects on mobility were not correlated with HLB and (v) effects on solute mobility were not always obtained instantaneously.[20,21] With increasing size (molecular mass) of surfactants, more time was needed until maximum effects were observed. This indicates that surfactants must penetrate into cuticles before they can exert an effect on solute mobility. From these studies it was suggested that surfactants are sorbed into cuticular waxes and increase their fluidity. As a consequence, the mobility of solutes sorbed in these fluidised amorphous cuticular waxes are increased. This was confirmed by recent work with reconstituted waxes where wax/water partition coefficients for monodisperse ethoxylated alcohols and the effects of these surfactants on mobilities of pentachlorophenol and tetracosanoic acid in isolated cuticlar waxes were measured.[23,24] Surfactant effects on diffusion coefficients (D) in the wax of these two compounds increased with increasing amounts of surfactants sorbed in the wax. Using ESR spectroscopy it was shown that the fluidity of cuticular waxes increased in the presence of those sorbed surfactants, which acted as plasticisers.

Sorption of ethoxylated alcohols and free alcohols from aqueous solutions into isolated cuticles follows simple laws.[25] Cuticle/water partition coefficients, the CMC and amount of surfactant sorbed can each be calculated using a set of linear equations from the number of carbon atoms in the alcohol and the number of the ethoxy groups, respectively. With this information, the amounts of surfactant in cuticles in the previous UDOS experiments,[20,21] studying effects of fatty alcohols and ethoxylated alcohols on mobility of 2,4-D in *Citrus* leaf cuticular membranes, were calculated. The dose-effect curve obtained was linear, showing that the surfactant effect was non-specific and depended only on the amounts of surfactant sorbed in the cuticles. Thus, the number of the carbon atoms in the alcohol and the number of ethoxy groups affected the CMC, the partition coefficient and rates of penetration, leading to differing amounts sorbed, but did not affect the intrinsic effectiveness of the individual homologues.

Adjuvants which increase solute mobility in cuticles and cuticlar waxes have been termed accelerator adjuvants. As already mentioned, ethoxylation is not required for accelerator action, as fatty alcohols can be powerful accelerators.[21] Since fatty alcohols can be surface-active there is still a chance that surface activity might be necessary. However, many other compounds which are not surface-active (Baur, P., Schönherr J., unpublished results) including chlorfenvinphos[26] also exhibit strong accelerator activity, showing that surface activity is not essential. Chlorfenvinphos was found to be a very effective accelerator and its own mobility in pear leaf cuticles was strongly concentration-dependent.[26] A high volume fraction of chlorfenvinphos was accompanied by a decrease of the activation energy of diffusion and the compound thus behaved in a similar way to external plasticisers of polymers. Which

property exactly imparts good accelerator activity to a compound is not known at this time, but the compound must be soluble in cuticlar waxes, since effects increase with increasing concentration in cuticular waxes.[24–26] A liquid state and lipophilicity alone are not sufficient prerequisites.[26]

The above results obtained using isolated cuticles and reconstituted waxes help to understand better adjuvant action on solute mobility in cuticles and on the permeability of cuticles. However, rates of uptake from droplets and spray residues depend on permeability and driving forces, just as electrical current depends on conductivity and potential gradient. If there is no potential gradient, no current will flow no matter what the conductivity is. The same holds for foliar uptake. Slow penetration does not necessarily imply low permeability of cuticles. It could result from small driving forces. This aspect has been discussed recently by Schönherr and Baur[13] and Baur and Schönherr[27] who also pointed out that the aspect of driving forces has generally been neglected in earlier investigations, where only biological effects or rates of penetration have been measured. If droplets of radio-labelled active ingredients are applied to leaves, only the rates of disappearance from the surface can be measured, while driving forces (which vary with time) remain unknown and their effects on the rates of uptake are usually neither discussed nor acknowledged.

Stock *et al.*[9] studied uptake into leaves of model compounds differing in molecular weight and partition coefficients with particular reference to effects of ethoxylated alcohols differing in numbers of ethoxy groups. Small droplets (0·2 μl) were applied and disappearance from the surface residue was monitored at various time intervals. Rates of uptake of the polar methylglucose were best enhanced by surfactants having 15 or 20 ethoxy groups, while rates of uptake of the lipophilic permethrin were highest when only six ethoxy groups were attached to the C_{13}/C_{14} alcohols. From the data, a three-dimensional qualitative model was derived which related rates of uptake to surfactant ethoxy content and octanol/water partition coefficient of the model compounds. This model was the first systematic approach to show a qualitative relationship between the nature of the penetrant and that of the accelerator adjuvant required for optimal penetration across a wide spectrum of lipophilicity (log K_{ow} -3 to $+6$) of penetrants. The paper hinted that other factors such as the role of water sorption in the surface residue, molecular mass of the penetrant, the nature of the cuticle, the pH for ionisable penetrants, etc. would be involved in influencing the actual rates of penetration of active ingredient and adjuvant. However, since the method of application was to place deposits (which dried) on the leaves attached to plants, the effect of the surfactant adjuvant on the concentrations (driving forces) of the compounds penetrating was an unknown factor and this prevented true kinetic and thermodynamic treatment of the data, which might have aided understanding of the process at the molecular level. In order to separate these effects we have used a similar set of model compounds and similar polydisperse surfactants and have studied their effects on solute mobilities in, and penetration across, isolated cuticles and related these to stationary surfactant concentrations in cuticles and cuticular waxes.

2 MATERIALS AND METHODS

2.1 Cuticular membranes

Adaxial astomatous cuticular membranes were isolated enzymatically from mature leaves of bitter oranges (*Citrus aurantium* L.) and pear (*Pyrus communis* L. cv. Bartlett) in 1991 and 1992 as described elsewhere.[28] Bitter orange plants were grown in growth chambers while pear leaves were taken from trees in an orchard in Bavaria in July. Isolated cuticles were obtained from these leaves by enzymatic degradation as described previously, air dried and stored for at least four weeks at about 8°C prior to use. During this initial storage of isolated cuticles, their water solute permeabilities decrease somewhat, which might be induced by structural rearrangement of cuticular waxes and/or loss of plasticising volatiles from cuticles.[29] After about four weeks' storage, permeabilities of cuticles become more constant and time independent.

2.2 Chemicals

All transport experiments were carried out with ^{14}C-labelled compounds (Table 1) which, in the following, will be referred to as solutes. They were stored as solutions in recommended solvents in the refrigerator. Before use the solvents were removed by a gentle stream of nitrogen and the compounds redissolved in different solvents containing mainly water (see Section 2.3). Applied concentrations varied in the experiments between 0·07 (cyanazine) and 1 mM (permethrin).

The surfactants studied were polydisperse fatty alcohol ethoxylates (alkyl-ω-hydroxypoly (oxy-1,2-ethanediyl)) of the 'Genapol' (GP) C series (Hoechst AG, Frankfurt, Germany). All surfactants had the same distribution of the alkyl chain length (Fig. 1A) the main constituent of the alkyl moiety being dodecanol (48 to 58%) followed by tetradecanol (19 to 24%), hexadecanol (9 to 13%), octadecanol (5 to 9%) and decanol (0 to 3%) (data from Hoechst AG, Frankfurt). The weighted mean number of carbons in the alcohols was 12·5. The distribution of the number of ethoxy groups (E) per alcohol together with the calculated mean value are given in Fig. 1A. The mean value resembles the weighted mean of all E derivatives (all alkyl chain lengths). The frequency distribution of E number per

TABLE 1
List of Radiolabelled Test Compounds

Compound	*Chemical name*	*Specific activity* ($MBq\ mmol^{-1}$)	*Radiochemical purity* (%)
Phenylurea[a]	[*Carbonyl*-^{14}C]phenylurea	148	99
2,4-D[b]	2,4-Dichlorophenoxy-[1-^{14}C] acetic acid	329	>98
Cyanazine[a]	2-(4-Chloro-6-[1-^{14}C]ethylamino-1,3,5-triazin-2-ylamino) -2-methylpropionitrile	507	99·5
WL110547[a]	1-(3-Fluoromethylphenyl)-5-[U-^{14}C]phenoxy-5*H*-1,2,3,4-tetrazole	503	99
Chlorfenvinphos[a]	2-Chloro-1-(2,4-dichloro-[U-^{14}C]phenyl)vinyl diethyl phosphate	303	99
Permethrin[a]	3-phenoxybenzyl (1*RS*)*cis*, *trans*-3-(-2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	68	96·7

[a] Shell Research Centre, Sittingbourne, UK.
[b] Sigma Chemie, Deisenhofen, Germany.

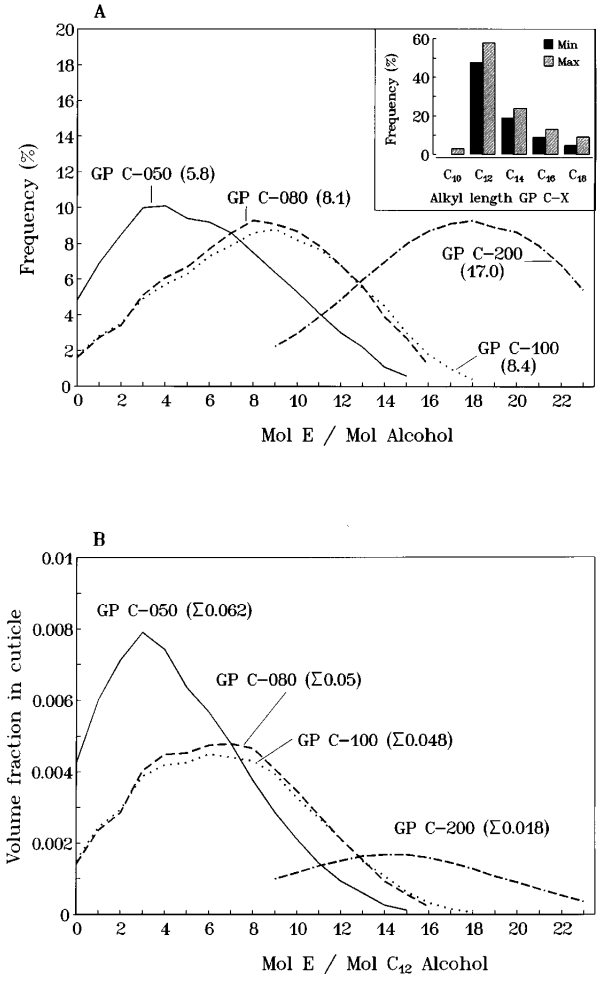

Fig. 1. (A) Ethylene oxide (Mol E) content per alcohol homologue and alkyl length distribution (see inset) of fatty alcohol ethoxylates of the 'Genapol' C series. The figure in brackets gives the weighted mean number of ethoxy groups. (B) Volume fractions of C_{12} ethoxylate homologues in the cuticle equilibrated with a micellar aqueous solution of the respective surfactant. The figure in brackets gives the total volume fraction for each surfactant.

alcohol is very broad and does not obey the Poisson distribution which is often found for nonionic surfactants. A Poisson distribution is obtained from acid catalysis, while alkaline conditions result in a broader distribution.[30] The surfactants contained 1·6 (GP C-080/100) to 4·8 (C-050)% free alcohols and 2·2 (GP C-050) to 3·5 (C-100) unidentified impurities. GP C-050 was liquid at room temperature while GP C-080 and GP-C100 were pastelike and GP-C200 was a waxy solid. All surfactants were free of water (<0·5%) and were stored at 4°C. The pH of 0·5% solutions varied between 5·7 (GP C-050) and 6·0 (GP C-200).

In all transport experiments a 10 g $litre^{-1}$ phospholipid (soybean lecithin) suspension (PLS) was used as control desorption medium. PLS does not change cuticlar permeability and does not measurably penetrate into the CM[22,31] but serves as a sink for permeated lipophilic compounds owing to its sorption properties.[17,32,33]

2.3 Experimental

2.3.1 Unilateral Desorption from the Outer Surface (UDOS)

Mobilities of compounds were determined by unilaterally desorbing radiolabelled solutes contained in the CM from the outer surface (UDOS). Details of the method have been described elsewhere[17,22] and only the principles and modifications will be described here. Cuticles were inserted between the lid and the desorption chamber with the morphological outer surface facing the chamber interior. The donor solutions containing the radiolabelled compounds were applied as 50-μl droplets to the centre of the morphological inner surface of the CM. It is essential in these UDOS desorption experiments that, during evaporation of solvents (3–6 h), the compounds are quantitatively sorbed into

the cuticle to avoid solid residues being present on its inner surface. Owing to the different specific activities (Table 1) and physicochemical properties (Table 2) the donor solutions had to be modified for each compound. The amounts applied varied between 0·8 μg (cyanazine) and 20 μg (permethrin). WL110547 and chlorfenvinphos were applied in water containing 400 and 250 g kg^{-1} ethanol, respectively. Chlorfenvinphos theoretically has a sufficient water solubility, but alcohol was added because tiny droplets were visible on the bottom of the glass vial when dissolved in water only, indicating a slow dissolution process. 2,4-D is a weak acid with a pK_a of 2·73[34] and it was dissolved in 0·01 M lactic acid buffer adjusted to pH 3 with potassium hydroxide. Phenylurea and cyanazine were first dissolved in water and applied to *Citrus* cuticles. However, preliminary desorption experiments indicated that these compounds were not quantitatively sorbed in the cuticle, and scanning electron microscope (SEM) pictures showed solid residues on the inner surface of the CM.[35] In order to avoid solid residues of these compounds, polyethyleneglycol 400 (PEG 400) was added to the aqueous solutions because it is an involatile liquid and has enough solvent power for both compounds.[35] Amounts of 200 μg per CM were found to be optimum in preliminary experiments. PEG 400 keeps the compounds in a dissolved state and does not alter cuticular permeability. This was confirmed by adding PEG 400 to donor solutions of WL110547 which was easily sorbed into the CM (see Section 3). Similarly, even the lipophilic permethrin was not quantitatively sorbed into the *Citrus* CM during evaporation of the water and alcohol solvents. In this case inert phospholipid was added to the donor solutions. An amount of 400 μg per cuticle was chosen since desorption rates were maximum and the treated area was covered completely with this amount as shown by SEM.

After evaporation of the volatile solvents of the donor solutions, the chambers were closed by an adhesive tape on the donor side and desorption from the outer surface was started the next day by pipetting into the chamber a 10 g $litre^{-1}$ aqueous soybean lecithin suspension (phospholipid suspension, PLS) through a sampling port. For calculating rate constants (k^*) for UDOS data it is assumed that the concentration of the desorbed compound in the receiver is zero. This assumption is fulfilled if PLS is used as desorption medium for lipophilic neutral solutes which completely partition into the liposomes. During desorption the chambers were rocked horizontally while standing with the lids facing downward in wells of a thermostated (25°C) aluminium block. At predetermined intervals the desorption medium (PLS) was withdrawn and replaced by a fresh one. Desorption with PLS was carried out for two (*Pyrus*) to four (*Citrus*) days, and after it was continued with a micellar solution (5 g $litre^{-1}$) of the surfactants again between two and four days. After the last desorption step the CM were cut out and the residual radioactivity in the CM extracted using scintillation cocktail (Aquasafe 500, Zinsser, Frankfurt, Germany). Radioactivity in the desorption media and CM was assayed using a liquid scintillation counter (Packard CA 2000 counter, Downers Grove, IL, USA).

2.3.2 *Simulation of foliar uptake (SOFU)*

The whole penetration process from a solute/surfactant residue across the cuticle was simulated by applying droplets containing adjuvants and test compounds to the *outer* surface of CM followed by desorption from the inner surfaces of CM.

Simulation of foliar uptake (SOFU) was carried out with cyanazine and WL110547 as model compounds with moderate and strong lipophilic character, and *Pyrus* cuticular membranes. The amount of test compound was always approximately 1 μg and the donor volume was 5 μl which corresponds to concentrations of about 0·8 mM. The compositions of 'Genapol' surfactants are quite similar (see Fig. 1) and therefore only GP C-050 and GP C-200 were used at concentrations of 1, 2, 10, 20 g $litre^{-1}$, which is equal to amounts of 5 to 100 μg. Droplets with GP C-050 spread much more than those of GP C-200, with a slight dependence on concentration being observed. Attempts were made to cover equal areas but, particularly at higher concentra-

TABLE 2
Relative Molecular Mass (M), Molar Volumes (V_x), Octanol/Water Partition Coefficients, (K_{ow}) and Water Solubilities (S_{H_2O}) of Test Compounds

No.	*Compound*	*M*	V_x^a ($cm^3\ mol^{-1}$)	*log* K_{OW}	S_{H_2O} (25°C)[b] ($g\ litre^{-1}$)
1	Phenylurea	136	107	0·8	4·1
2	2,4-D	221	138	2·8	0·8
3	Cyanazine	241	177	2·1	0·17
4	WL110547	306	192	3·6	3×10^{-3}
5	Chlorfenvinphos	359	233	3·5	0·145
6	Permethrin	391	282	6·1	$0{\cdot}2 \times 10^{-3}$

[a] Reference 44.
[b] From Shell Research Ltd and for 2,4-D, Reference 34.

tions, the areas with GP-200 applications were still less than with GP C-050 due to the further spreading of GP C-050 some minutes after application. Control experiments were carried out by applying the compounds in 5 μl water or in aqueous PEG 400 (40 g litre^{-1}). The experimental conditions were as described above (Section 2.3.1). However, in contrast to the UDOS experiments, the chambers with cuticles were completely filled with desorption medium (PLS) and stood upright during the first 2 h with the sampling port being closed by adhesive tape. The bulk of the water in the 5-μl donor droplets had evaporated after 30–40 min leaving a liquid solute/surfactant residue. After the first sample (2 h), the adhesive tape closing the sampling port was removed and the chambers inverted so that they faced the aluminium block for the remainder of the experiment (24 to 33 h).

2.3.3 Calculation of rate constants describing diffusion (k^*) in and penetration (k) across cuticular membranes

In UDOS experiments, rates of diffusion were characterised by plotting the natural logarithm (ln) of the fraction of the applied test compound remaining *in* the CM against time. The ratio of the concentration C_t of the radiolabelled solute in the CM at time t to the original concentration C_0 at $t = 0$ decreases according to first-order kinetics.

$$\frac{C_t}{C_0} = e^{-k^* t} \quad (1)$$

with k^* being the first-order rate constant of desorption. The asterisk denotes that the driving force is the solute concentration *in* the cuticle and this rate constant (k^*) is therefore independent of solute lipophilicity as expressed by the partition coefficient K.[22] Plotting $-\ln(C_t/C_0)$ against t yielded straight lines with slope k^*. Since the volume of the CM in which solutes are sorbed in constant, the ratio C_t/C_0 is equivalent to the expression $1 - M_t/M_0$, where M_t is the amount of radiolabelled substance desorbed at time t, and M_0 the amount sorbed initially in the CM, which was calculated by summation of the amounts desorbed plus that remaining in the CM after termination of desorption. Because desorption was initially with PLS alone and then subsequently with surfactant solutions, two slopes were obtained and the ratio of desorption with surfactant to that with PLS was used to obtain a surfactant effect on mobility of the test compound.[17,20,21]

Equation (1) is only valid if the concentration of penetrants in the water of the receiver is zero. This is achieved through the good sorptive properties of PLS, which absorbs compounds differing in physicochemical properties into different regions of the vesicles.[32] Aqueous surfactant solutions above the critical micelle concentration can also solubilise lipophilic compounds into the interior of micelles.[36] The CMC of the Genapol surfactants used in this study differed only slightly about a value of $\sim$0·03 g litre^{-1} (25°C), as determined by the Wilhelmy plate method and was well below the lowest concentration used. Surfactant micelles would therefore have also acted as sinks for the permeated lipophilic test compounds.

In SOFU experiments, the droplet on the surface of the CM or the hydrated surfactant/solute residue remaining after evaporation of the bulk water served as donor. Radio-labelled solutes and surfactant molecules diffuse through the CM into the receiver solutions facing the inner surfaces of the CM. The whole process can be approximated by an equation similar to eqn (1), with the slopes of the SOFU penetration graphs being rate constants (k), though they are not equivalent to k^* obtained in UDOS experiments, where the solute concentration *in the cuticle* serves as donor:

$$\frac{C_t}{C_0} = e^{-kt} \quad (2)$$

The difference is that, while in UDOS experiments the donor volume remains constant during the experiment, this is generally not the case in SOFU since volatile solvents (water etc.) evaporate and both solutes and surfactants penetrate into the cuticle during this evaporation to an unknown extent leaving a surface residue. This means that there are at least two steps, in series, in the overall penetration process, i.e. firstly from the surface residue into the cuticular wax and, secondly, from the cuticle into the adjacent cell wall. The first step cannot be first-order since the volume, and hence the concentration of the penetrants, changes with time thus rendering the overall process not first-order. SOFU desorption graphs are only strictly first-order when penetration proceeds from a non-volatile and non-penetrating solvent on the surface of the cuticle under constant experimental conditions in humidity and temperature.[13,37] In this special case only, the concentration of the solute in the donor decreases exponentially with time, which results in linear graphs according to eqn (2). Between 10 and 50 CM (replications) were used for each species/compound/surfactant combination in UDOS and SOFU, respectively. Variability is expressed as 95% confidence intervals and is caused mainly by variability among individual CM.

3 RESULTS

3.1 Preliminary tests

3.1.1 Effect of the amount sorbed and areas of application on the rates of permeation in the UDOS method

Owing to different compositions of the donor solutions in UDOS, the treated area of the CM varied in these experiments. Rate constants of desorption should not

depend on the area if a compound is sorbed into the CM when applied to the inner side. In experiments with WL110547, a compound which causes no problems in UDOS experiments, rates of permeation using different donor volumes and concentrations of WL110547 and different treated areas were measured with *Citrus* CM (Table 3). No significant differences were found, in accord with expectations. In one experiment PEG 400 was added in a 50-μl droplet using the same amount as necessary for experiments with phenylurea and cyanazine. Again, no difference in rate constants was found (Table 3).

3.1.2 Effect of co-solvents on the rates of desorption in the UDOS method

As stated in Section 2.3.1, co-solvents had to be used either to prevent formation of solid residues of some of the compounds (phenylurea, cyanazine) or attain complete solution of permethrin during their application to *Citrus* CM. It was necessary to assess any effects these co-solvents, particularly the involatile PEG 400 and the phospholipid (PL), may have had on the rates of permeation. PEG 400 had no effects on desorption of WL110547 from *Citrus* CM (Table 3) and there were no effects of PEG 400 or PL on either the initial rates or the surfactant-enhanced rates of permeation of WL110547 through *Pyrus* CM in comparison with applications without the co-solvents, reported more fully in Section 3.3 (see Fig. 7).

3.1.3 Solubilisation of test compounds in surfactant micelles

Solubilisation of penetrated solutes in the surfactant micelles is necessary, not only to maintain the concentration in the aqueous phase at zero, but also to avoid precipitation of some very lipophilic and insoluble diffusants in the receiver solution. The lipophilic phase of 10 g litre^{-1} PLS and 5 g litre^{-1} micellar surfactant solutions (usually 0·65 ml) was generally in excess of the amount of diffused solute, with the exception of permethrin. In this case, owing to its low specific activity, desorption from *Pyrus* CM with 'Genapol' C-050 led to a diffused amount of up to 5 μg or $1{\cdot}8 \times 10^{-8}$ mol permethrin per day. This is close to the amount of surfactant, which is estimated to be 4×10^{-8} mol. Above the CMC the solvent power of surfactant solutions increases linearly with concentration, and we therefore used a 20 g litre^{-1} solution of GP C-050. However, in Figs 2A and 2C (which show typical plots for populations of CMs obtained in UDOS experiments—see Section 3.2), rates were maximum at the shortest time interval (asterisks in Figs 2A and 2C), suggesting that solubilisation may not have been complete at the longer intervals when more permethrin had penetrated. In all other experiments with the 'Genapol' surfactants and model compounds, solubilisation was not rate-limiting.[35]

TABLE 3
Rate Constants of Desorption of WL110547 from *Citrus* CM at Different Donor Volumes and Amounts per Area

Applied volume (μl)	*Area*[a] (cm^2)	*Amount per area* ($\mu g\ cm^{-2}$)	*Rate constant* k* $\times 10^{-7}$ (s^{-1}) ($\pm$95% *CI*)
5	0·13	2·65	2·4 ($\pm$1·0)
10 (2 × 5)	0·26	2·65	2·6 ($\pm$0·8)
50	0·5	6·7	1·9 ($\pm$0·8)
50	0·5	1·86	2·9 ($\pm$0·5)
100	0·95	0·98	3·5 ($\pm$1·1)
50 (+200 μg PEG400)	0·8	1·16	3·1 ($\pm$0·6)

[a] Maximum area just before complete evaporation of solvents.

3.2 UDOS experiments with *Citrus* CM

The effect of Genapol surfactants was investigated with all compound/surfactant combinations with *Citrus* CM. Typical plots of the time course of permeation of permethrin with GP C-050 at a concentration of 20 g litre^{-1} in the receiving solution for populations of CM in UDOS experiments obtained with *Citrus* and *Pyrus* CM are shown in Figs 2A and 2C. Initial rates of desorption with PLS (k^*_{PLS}) were determined during the first 65 h, in which the receiver solution contained only PLS. These rate constants represent solute mobilities in cuticles before treatment with surfactant. On substitution of the PLS solution by a receiving solution containing 20 g litre^{-1} GP C-050, the rates increased markedly, but it took between 20 and 60 h before desorption plots became linear again. During this time surfactants penetrate into the CM and their concentrations in CM and cuticular waxes increase with time until equilibrium between micellar surfactant solutions and the CM is obtained.[20] The surfactant-enhanced rates of permeation were calculated from the subsequent linear portion of the plots, in this case, generally at the shortest interval between 112 and 119 h. As always observed in this type of study there was a large variation in initial desorption rates between the CM samples. Furthermore, as also observed previously, the greatest rates of enhancement were with those CM samples that gave the slowest initial rates as demonstrated in a plot of maximum effect ($k^*_{GP\ C\text{-}050}/k^*_{PLS}$) against $1/k^*_{PLS}$ (Figs 2B and 2D), and this relationship is reasonably linear. The consequence is that the surfactant enhancement tended to reduce differences in solute mobilities between the CM.

CM having very low k^*_{PLS} values may never reach cuticle/surfactant sorption equilibrium, as seen in Figs. 2A where the lower four desorption graphs were not linear even after 165 h. In these cuticles permethrin

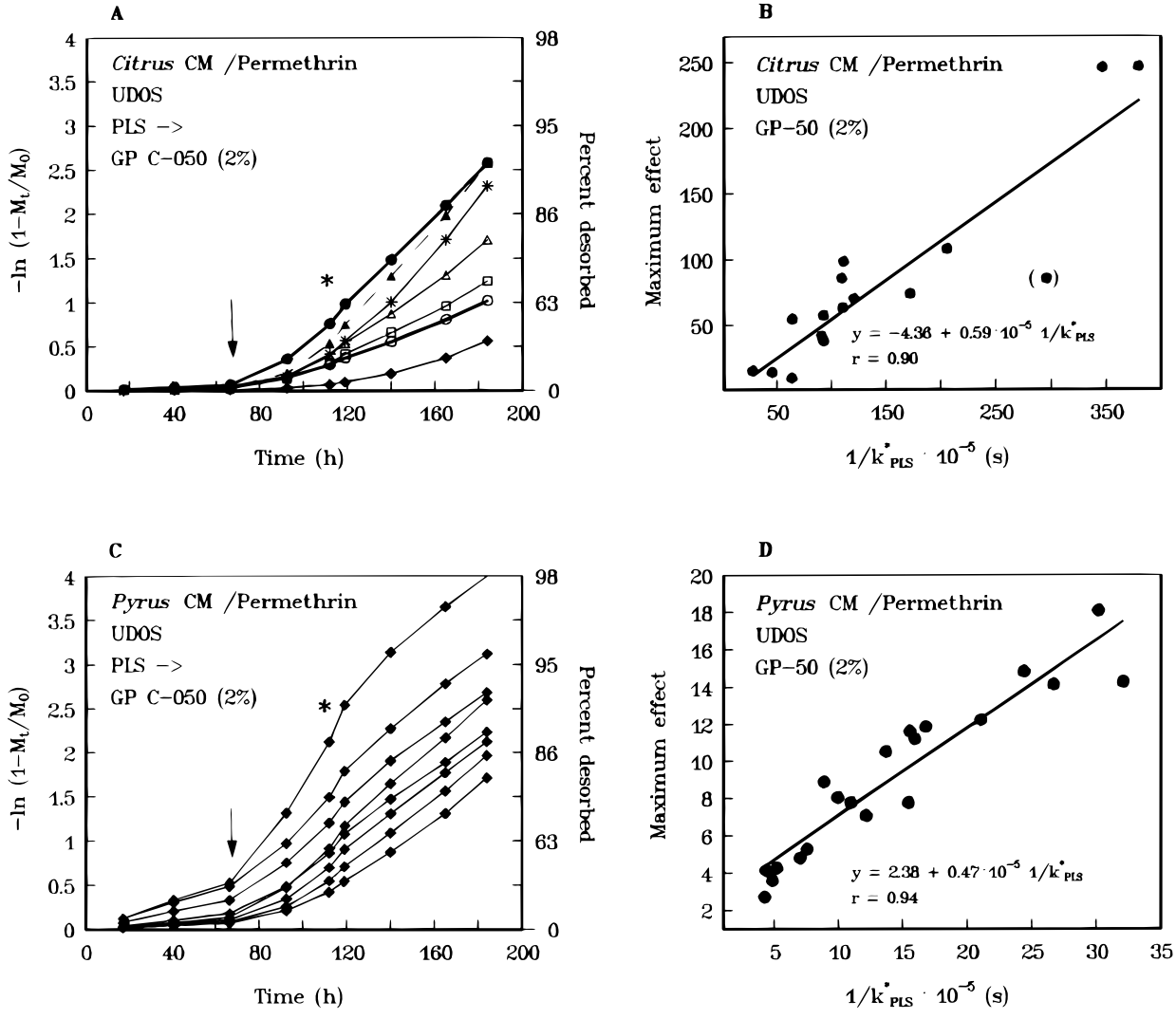

Fig. 2. (A) Time course of desorption of permethrin from *Citrus* CM with PLS or (indicated by the arrow) an aqueous solution of 'Genapol' C-050. The lines represent desorption from seven individual CM. (B) Dependence of the maximum effect on initial rate constants measured using PLS as desorption medium (k^*_{pls}). The maximum effect is the ratio of maximum rate constants of desorption (in this case between 112 and 119 h, see asterisk) over k^*_{pls}. This ratio was calculated for each CM separately. (C) and (D) show the analogous results for *Pyrus* CM (see text).

mobility and surfactant penetration were very low, such that sorption equilibrium and maximum surfactant effect were not always obtained. In the response graphs (maximum effects versus $1/k^*_{PLS}$) this results in a deviation from linearity, the data point in brackets (Fig. 2B) lying far below the line fitted to the data.

Similar plots (not shown) and rates (Table 4) were obtained for all the compound/surfactant combinations. Solute mobilities (k^*) declined by a factor of 52 when molar volumes increased 2·64-fold (column 1, Table 4 and Table 2). The mean surfactant enhancement effects generally *in*creased through the series of compounds (i.e. with increasing molar volumes of solutes) but *de*creased with increase in extents of ethoxylation of the surfactants from GP C-050 to GP C-200. In fact there were negligible enhancements by GP C-200 for all compounds with *Citrus* CM. A three-dimensional bar chart of the log[mean effect] for each surfactant, at a concentration of 5 g litre^{-1} in the receiver solution, against the molar volume of the compounds clearly shows the systematic trends (Fig. 3). The dependence of surfactant effects on initial rate constants of desorption was obtained for all compounds only with GP C-050. Coefficients of correlation were smaller with *Citrus* than observed with *Pyrus* CM, with the highest value of $r^2 = 0{\cdot}81$ for permethrin (Fig. 2B).

3.3 UDOS experiments with *Pyrus* CM

Studies with *Pyrus* CM were more limited than those with the less permeable *Citrus* CM. Only WL110547 was used with all 'Genapol' surfactants and only GP C-050 with the range of compounds.

The effects of the 'Genapol' surfactants with WL110547 were that solute mobilities increased after

TABLE 4
Rate Constants of Test Compounds (k^*) and Effects of 'Genapol' Surfactants on Mobilities of Test Compounds in *Citrus* cuticular membranes

Compound	k* × 10^{-7} (s^{-1})[a] (±95% *CI*)	*Mean effect* (±95% *CI*) *Genapol*			
		C-050	*C-080*	*C-100*	*C-200*
Phenylurea	24·6 (±8·5)	2·9 (±0·77)	2·13 (±0·38)	1·36 (±0·20)	—
2,4-D	6·76 (±2·1)	6·8 (±1·62)	2·80 (±0·40)	1·60 (±0·15)	—
Cyanazine	4·37 (±1·5)	6·4 (±1·31)	1·58 (±0·61)	1·55 (±0·17)	—
WL110547	4·07 (±1·4)	15·2 (±3·8)	5·01 (±0·90)	2·91 (±0·65)	—
Chlorfenvinphos	1·12 (±0·6)	16·5 (±3·2)	5·40 (±0·80)	2·10 (±0·20)	—
Permethrin	0·47 (±0·1)	82·1 (±35)	13·35 (±9·04)	2·38 (±0·29)	1·13 (±0·11)

[a] Arithmetic means and 95% confidence intervals were calculated from combined values of all experiments carried out with the respective compound.

changing the receiver solution from PLS to solutions of GP C-050, GP C-080 and GP C-100 (Figs 4A, B and C) but GP C-200 was barely effective (Fig. 4D). With GP C-050 the plots were curved over the first 48 h until the rates became constant, while with GP C-080 the plots were curved throughout the period (115 h) of the experiment but never reached the rates found with GP C-050. In contrast, the rates with GP C-100 were constant immediately after changing the receiver solution but were even slower than those with GP C-080. This was similar to that obtained for all compounds with *Citrus* CM. Enhanced rates of diffusion were reasonably inversely related ($r^2 = 0{\cdot}85$) to initial rates of diffusion ($1/k^*_{PLS}$) of WL110547 with GP C-050 but not with the other surfactants (Fig. 5). The regression line fitted to Fig. 5A was drawn into Fig. 5B and this reveals that all data points are below the line, that is, effects for a given $1/k^*_{PLS}$ were smaller with GP C-080 than for GP C-050. However, the plot of effects versus $1/k^*_{PLS}$ for the nine CM in the left hand corner (corresponding to those having the highest initial mobilities) is fairly linear, indicating that these CM reached or nearly reached sorption equilibrium with GP C-080, while those CM having lower WL110547 mobilities did not reach surfactant sorption equilibrium and maximum possible effects. This shows that surfactant effects on solute mobilities in cuticles is variable and dependent on the cuticle. If surfactant permeability of CM is low, potential maximum effects may never be realised, as with GP C-100 (Fig. 5C).

The effect of GP C-050 on the range of compounds was to increase their rates of diffusion markedly, with

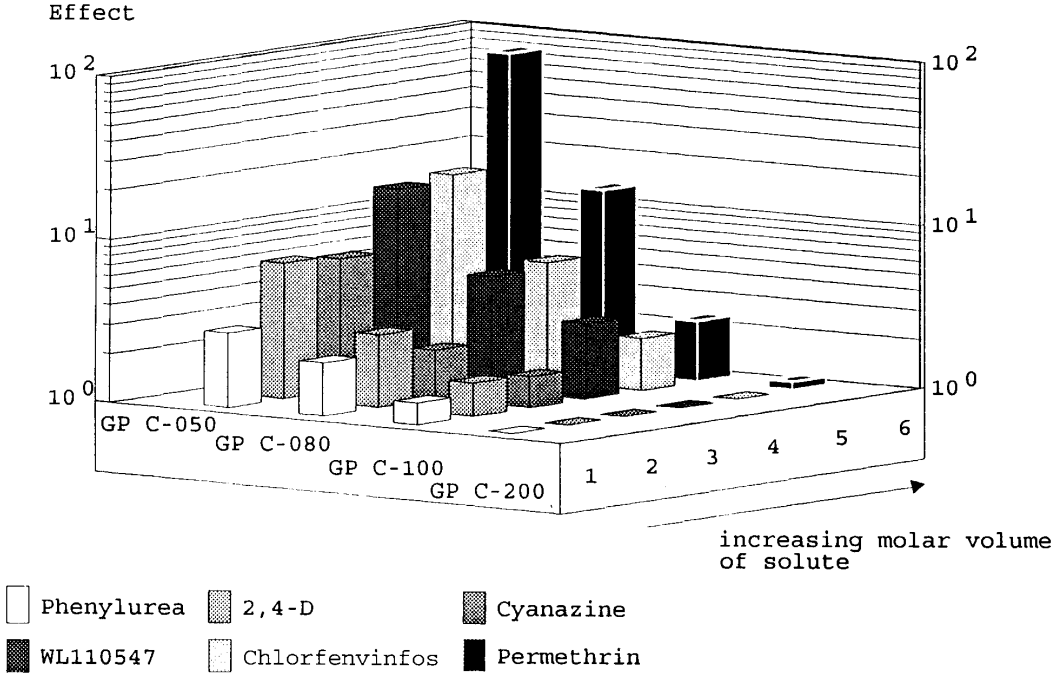

Fig. 3. Mean effects of 'Genapol' C surfactants on mobilities of the model compounds in *Citrus* CM.

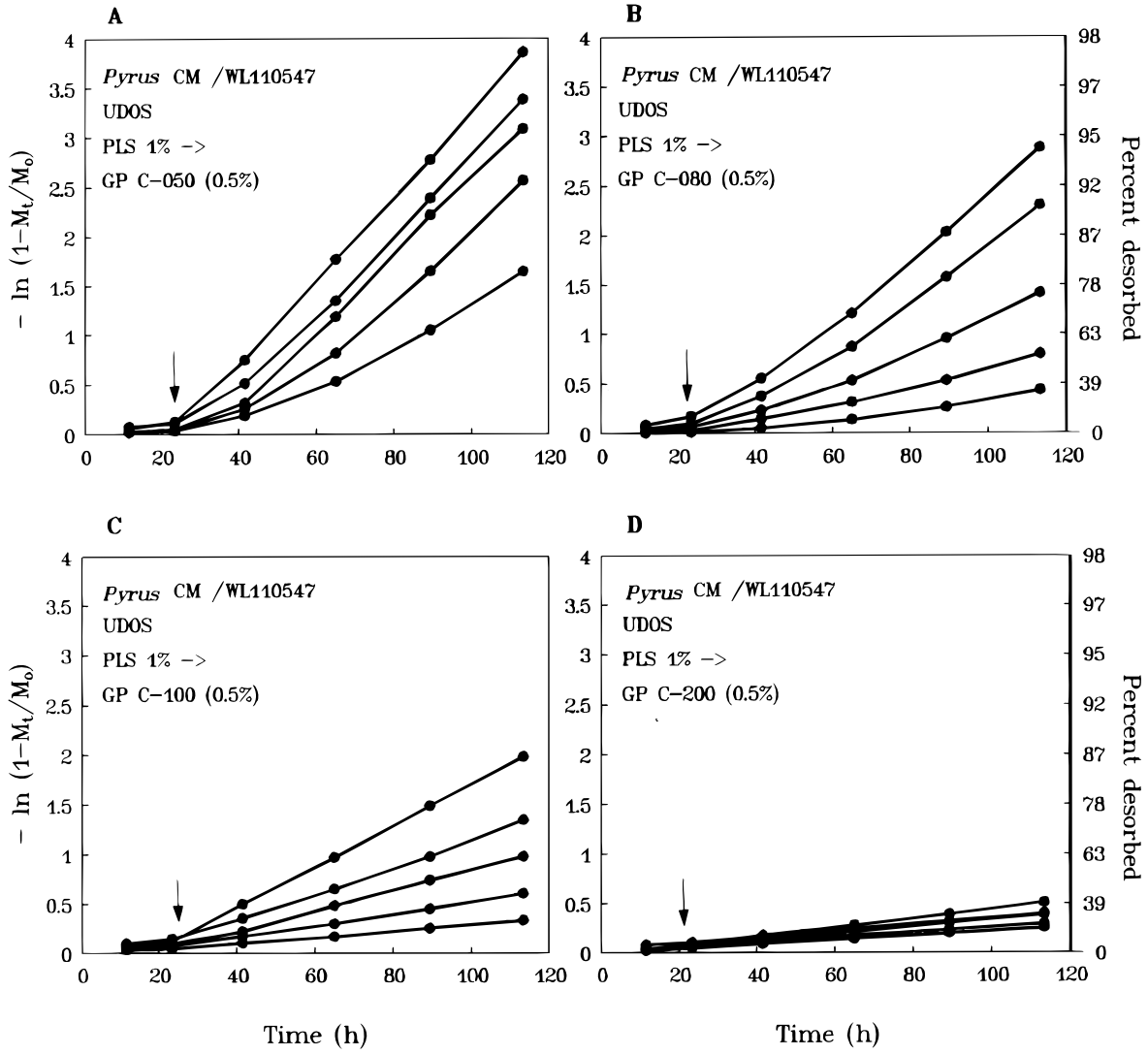

Fig. 4. Time course of desorption of WL110547 from *Pyrus* CM with PLS or (indicated by the arrow) an aqueous solution of 'Genapol' C surfactants. The lines represent desorption from five individual CM.

curvature of the plots during the first 48 h after substitution of the PLS receiver solution by GP C-050 solution for all compounds (Fig. 6). Curvature depended strongly on the initial mobility in the CM (Fig. 6B), where the graph for the CM with high 2,4-D mobility showed an instantaneous increase in slope after changing from PLS to GP C-050, while the slope for the CM with the lowest 2,4-D mobility increased throughout the period. The effect of GP C-050 varied with $1/k^*_{PLS}$ of the *Pyrus* CM for all model compounds (Fig. 7).

In order to assess the effect of the co-solvents necessary for desorption experiments with phenylurea, cyanazine and permethrin with *Citrus* CM, some of the data were obtained from applications made in the absence and presence of either PEG 400 (phenylurea, cyanazine) or PL (permethrin). There were no discernible differences between these (Fig. 6) and thus no effect of these co-solvents on the rates of diffusion, either initially (Fig. 6) or subsequently (Fig. 7) on the enhanced rates induced by GP C-050. These GP C-050 enhanced rates also correlated with the initial rates of diffusion (Fig. 7), again showing that the increase was greatest for the initially least permeable CM.

3.4 SOFU experiments with *Pyrus*

If WL110547 and cyanazine were applied in water or in an aqueous solution with PEG 400, the amounts which had penetrated the *Pyrus* CM 24 h after application were, at best, only 15% of the amount applied. Addition of surfactants to the application solution increased the rates of uptake markedly compared with the controls without surfactant (Fig. 8A). SOFU plots (ln [amount in the surface residue] against time) in the presence of GP C-050 were convex (i.e. did not follow first-order kinetics) for all amounts of GP C-050 with WL110547. Slopes increased with increasing amounts of GP C-050 and, even though slopes decreased at later stages, the slopes for the two highest surfactant amounts (50 and

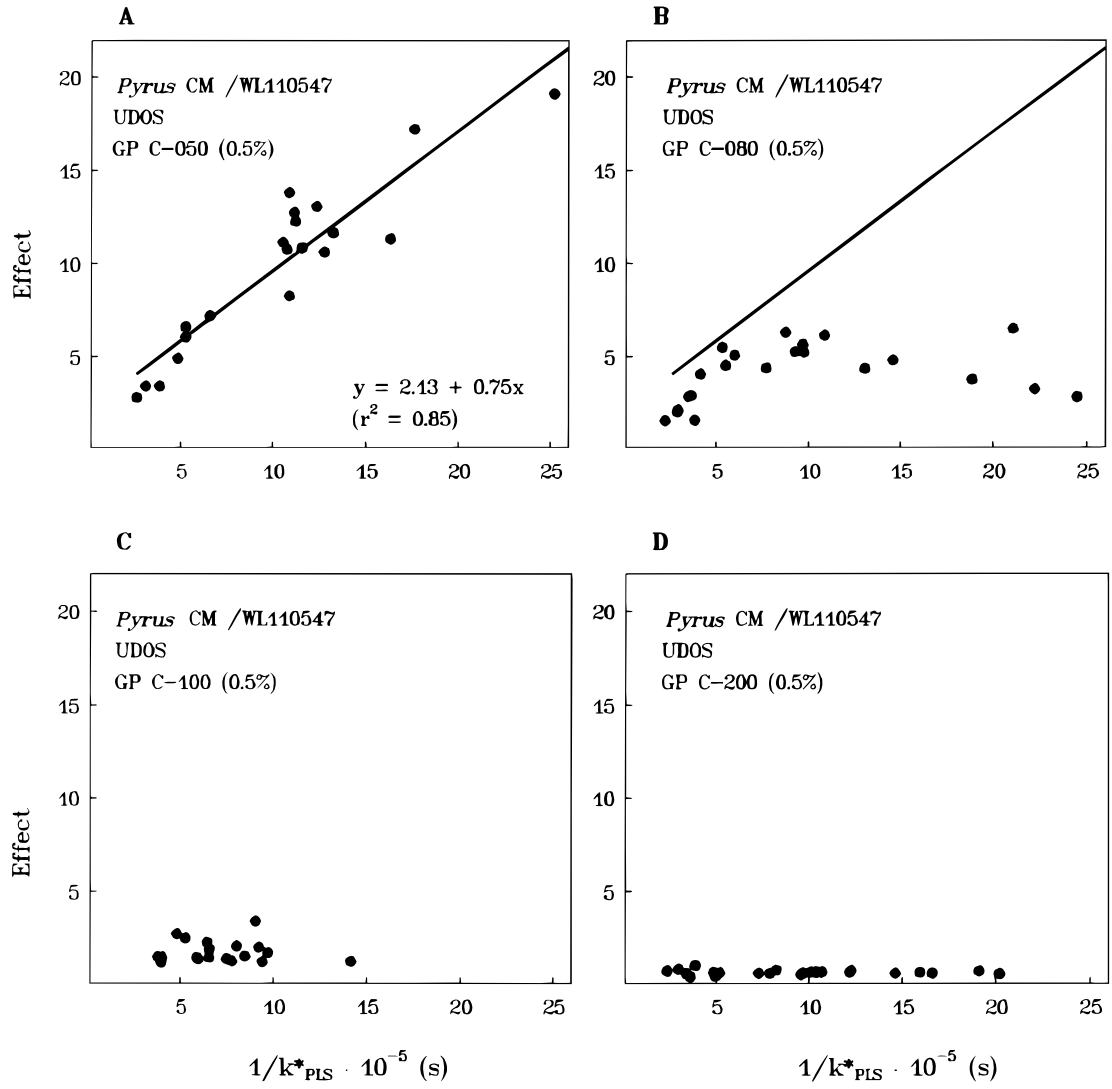

Fig. 5. Dependence of the effect of 'Genapol' C surfactants on initial rate constants of desorption of WL110547 from *Pyrus* CM.

100 μg) remained higher than those with the lower amounts. As a consequence, only 45–55% of WL110547 applied penetrated in the presence of 5 and 10 μg, while 88–91% penetrated in the presence of 50 and 100 μg surfactant, respectively. Similar plots with GP C-200 were linear, increasing with increasing amounts of GP C-200 up to an optimum amount of 50 μg. Rates of penetration of WL110547 were much smaller at all times when applied together with GP C-200 than if applied together with GP C-050 (Figs 8A and B).

The more polar cyanazine penetrated somewhat more slowly than the lipophilic WL110547 (Fig. 9), but the effects of the two surfactants on the rates of penetration were similar. The enhanced penetration of cyanazine in the presence of GP C-050 increased with increase in the amount of GP C-050 applied up to 50 μg but, with 100 μg surfactant, rates were smaller than with 50 μg (Fig. 9A). Slopes were steepest initially (up to 6 h) but became approximately linear thereafter. The enhancement of the penetration rates of cyanazine by GP C-200 was small at all amounts applied, being less than with WL110547 and GP C-200 (cf. Figs 9B, 8B). Both sets of rates were approximately linear and varied little with the amount of GP C-200 applied. No surface residue of GP C-050 was visible with the lower amounts applied (5 and 10 μg) after the third sample (20 h) while with GP C-200 residues could be seen throughout the entire experiment.

4 DISCUSSION

4.1 Solute and surfactant flows in UDOS and SOFU experiments

Unilateral desorption from the outer surface (UDOS) provides three sets of information: (i) effects of molar volumes of solutes and temperature on mobility in CM, (ii) effects of surfactants on solute mobility in CM and (iii) velocity of penetration of surfactants into CM as seen from the time required to realise maximum surfactant effects. In UDOS experiments the majority of

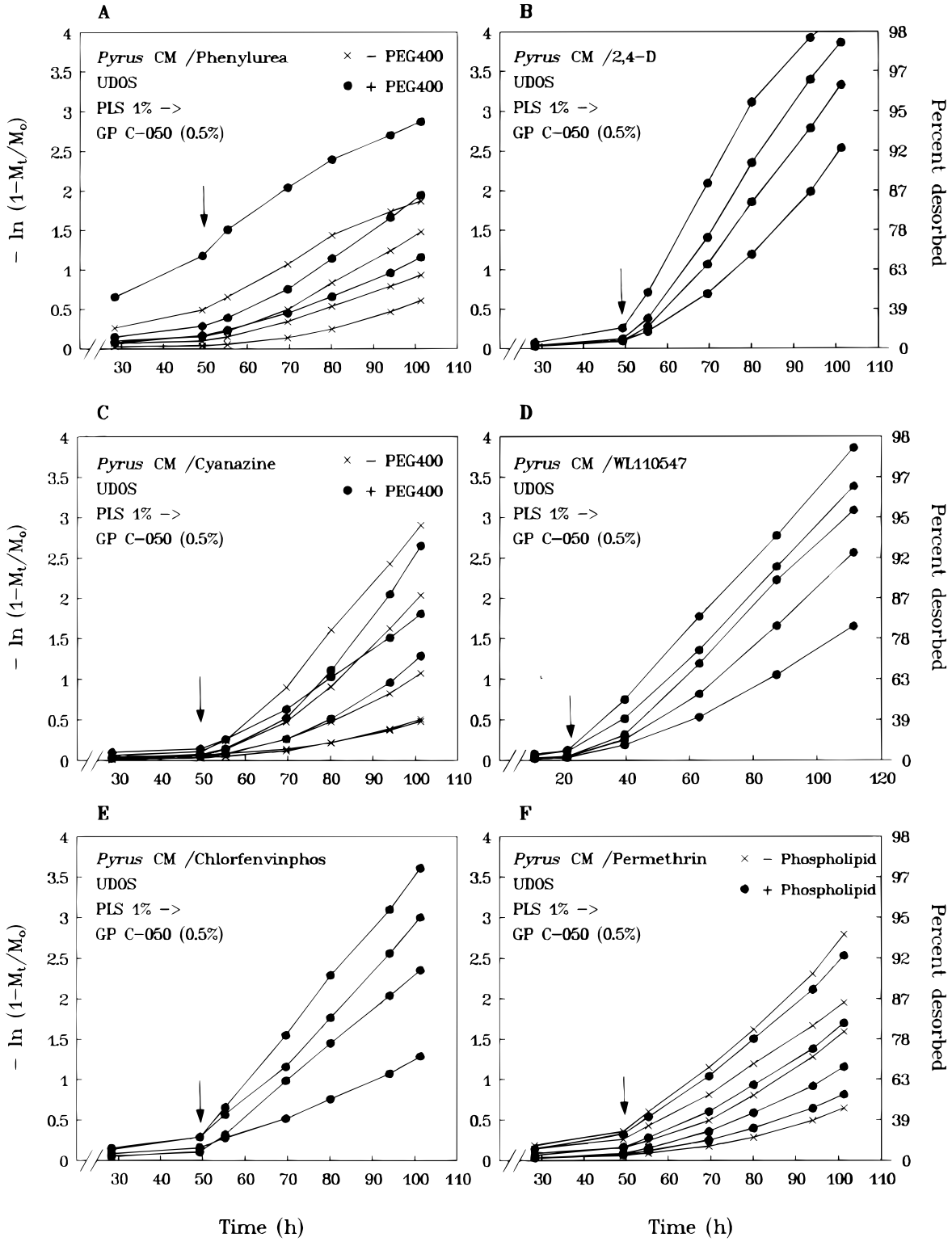

Fig. 6. Time course of desorption of the model compounds from *Pyrus* CM with PLS or (indicated by the arrow) an aqueous solution of Genapol C-050. The lines represent desorption from four to eight individual CM (see text).

radio-labelled solute molecules are initially contained in the sorption compartment of the cuticles[17,22] (Fig. 10), because the sorption compartment amounts to about 90% of the total volume of the CM[37] and apparent partition coefficients of organics in cutin are at least 10 times higher than in cuticular waxes.[23,24,39] When the receiver solution is brought into contact with the outer surface of the CM, solutes start to diffuse from the sorption compartment across the waxy limiting skin into the receiver solution. Rates of diffusion are determined by

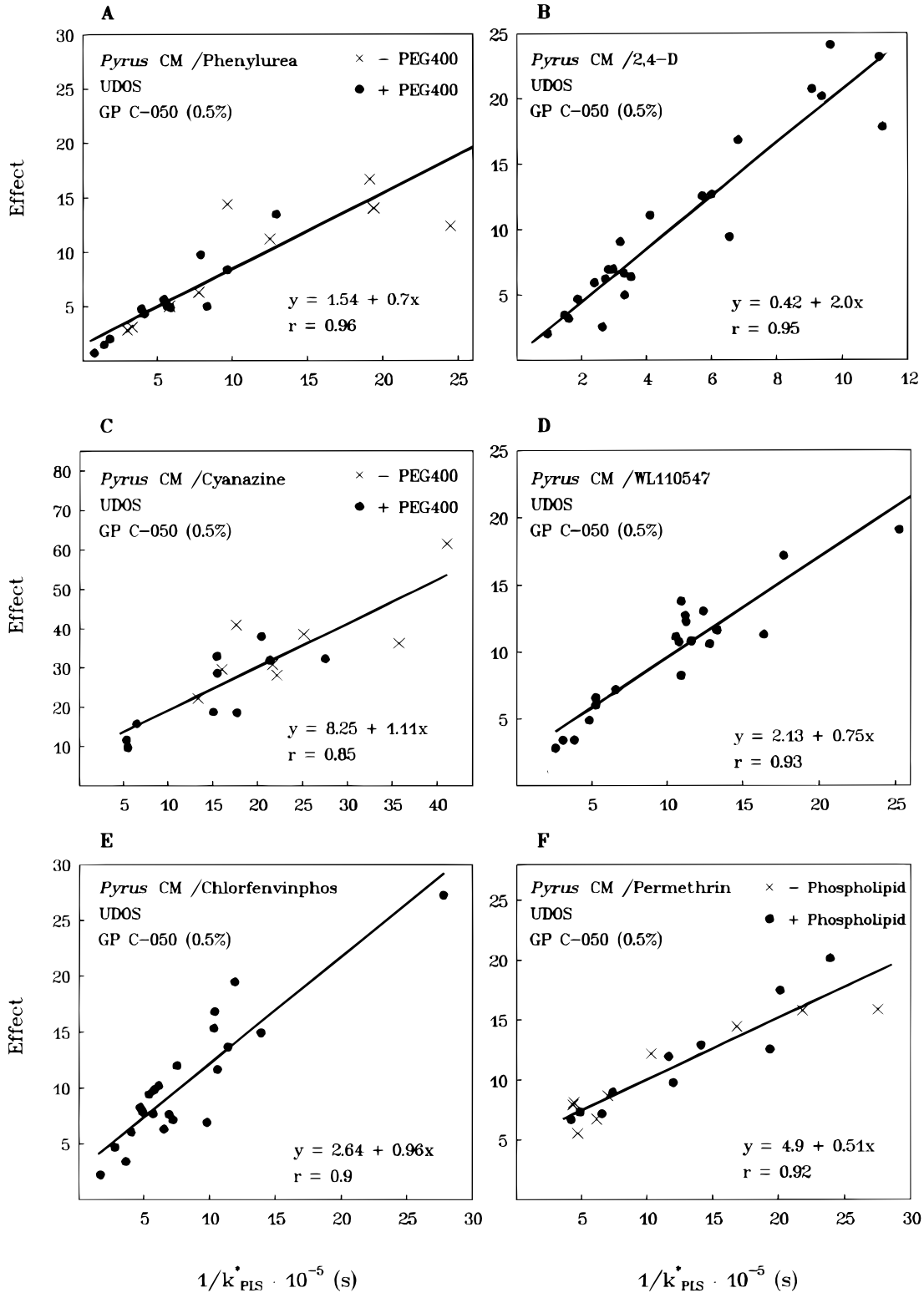

Fig. 7. Dependence of the effect of 'Genapol' C-050 on initial rate constants of desorption of the model compounds from *Pyrus* CM.

solute mobility in the waxes of the limiting skin. Estimating solute mobility in the limiting skin requires that desorption media do not contain compounds which penetrate the CM and change the structure of cuticular waxes. If the desorption media contain relatively small and lipophilic surfactants which can penetrate the cuticle and are sorbed in cutin and cuticular waxes, the cuticles will be loaded with surfactants until sorption equilibrium between micellar solutions and CM is obtained.[20–25] This loading with surfactants proceeds

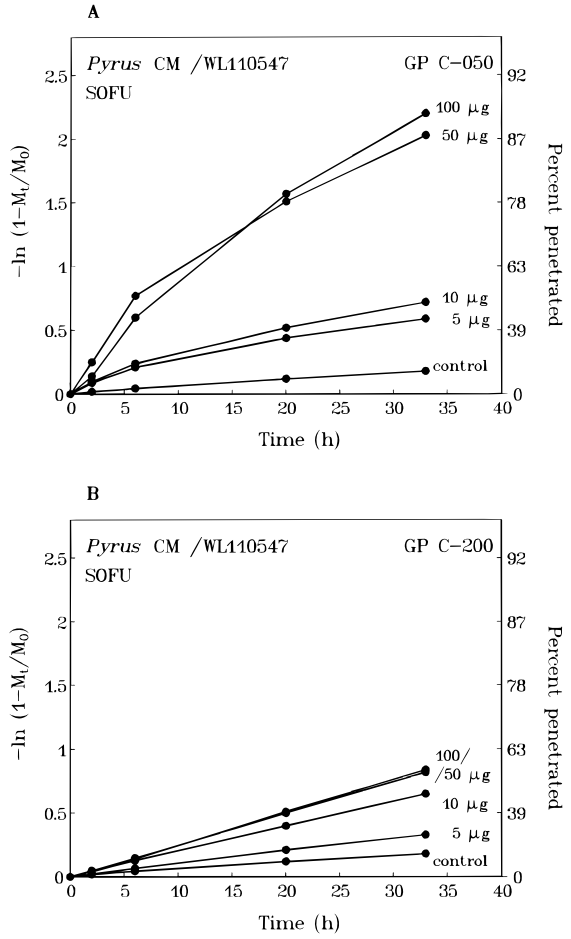

Fig. 8. Simulation of foliar uptake of WL110547 across *Pyrus* CM as influenced by different amounts of the surfactants (A) 'Genapol' C-050 and (B) C-200 (means of eight to 14 CM).

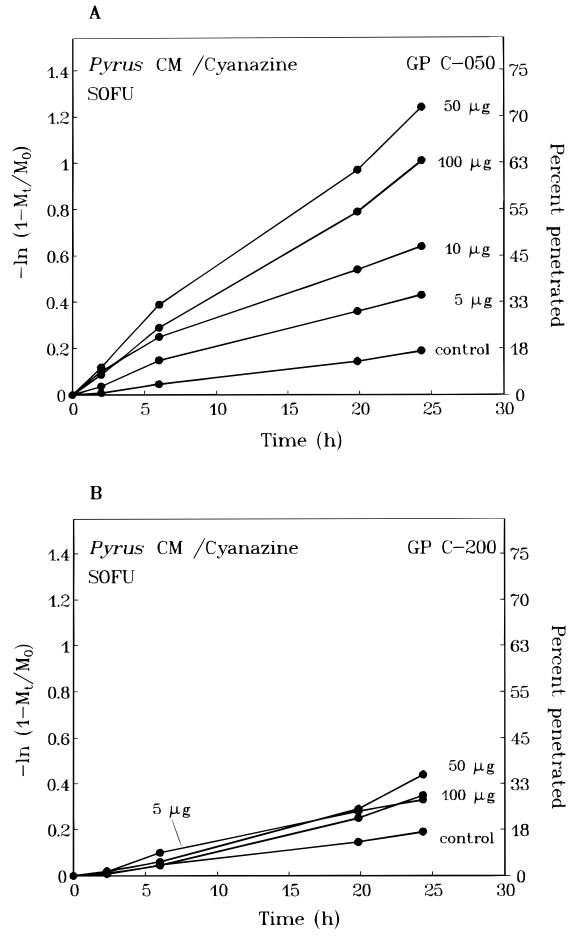

Fig. 9. Simulation of foliar uptake of cyanazine across *Pyrus* CM as influenced by different amounts of the surfactants (A) 'Genapol' C-050 and (B) C-200 (means of six to 13 CM).

from large volumes of micellar surfactant solutions which are repeatedly exchanged with fresh ones when samples are taken. Surfactants cannot escape from the CM as there is no solution in contact with the inner surface of the CM (Fig. 10). The time required for equilibrium depends on the mobility of the surfactant in the limiting layer of the CM, that is on the molar volume of surfactant molecules, just as with other molecules.[13,22] However, accelerators like $C_{12}E_8$ or chlorfenvinphos are much more mobile than other compounds of comparable size.[26] The concentrations of ethoxylated alcohols sorbed in cuticles when in equilibrium with micellar solutions decreased with increasing numbers of carbon atoms (*nC*) in the alcohol and the number of ethoxy groups (*nE*)[25]

$$\log C_{surf} = 2.97 - 0.02nC - 0.08nE \qquad (3)$$

where the surfactant concentration (C_{surf}) has the dimension mmol kg^{-1}. This equation has been derived using monodisperse ethoxylated alcohols. Sorption of polydisperse surfactants in cuticles can be obtained by calculating sorption for each homologue separately and summing up. This has been done for the polydisperse Genapol surfactants using C = 12 and the number of ethoxy groups given in Fig. 1A. In Fig. 1B volume fractions of surfactant homologues have been plotted for each polydisperse mixture. The total concentration of $C_{12}E_x$ in the cuticle at equilibrium corresponds to the area under each curve and is given in parenthesis. The actual volume fraction is even greater since the C_{14}—and other homologues are not included in the values given in Fig. 1B, but the differences between the Genapols still exist. As predicted by eqn (3) the amounts sorbed decrease with increasing ethoxylation both for the individual homologues and the entire mixtures. With increasing ethoxylation the composition of the surfactants sorbed in the cuticles changes towards more polar homologues and the total amount sorbed decreases, such that 3·44 times more GP C-050 was sorbed than GP C-200. Still, a significant amount of surfactant would be sorbed in cuticles at equilibrium even from GP C-200 micellar solutions.

It is an important feature of UDOS experiments that, given enough time, sorption equilibrium between surfactant micelles and cuticles is obtained, such that surfactant-enhanced solute mobilities can be related to stationary surfactant concentrations in the cuticle and in cuticular waxes.

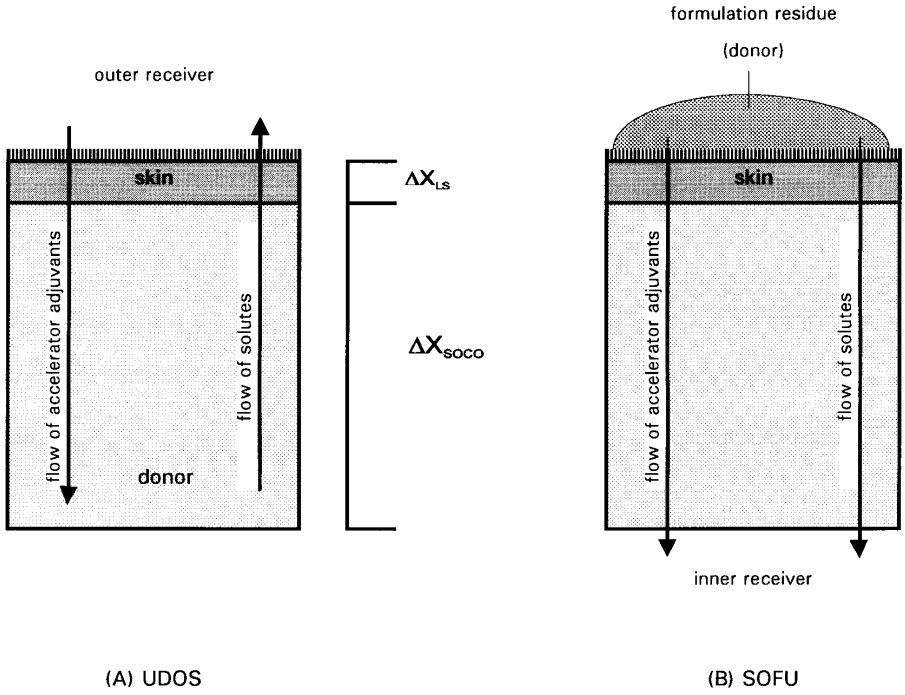

Fig. 10. Schematic drawing of the cuticle and the directions of solute and surfactant flow in (A) UDOS and (B) SOFU (from Ref. 13).

The data of Fig. 1B refer to sorption in cuticles, and most of the surfactants will be associated with the cutin of the sorption compartment. By comparing partition coefficients of monodisperse alcohol ethoxylates obtained for the systems isolated wax/water and polymer matrix/ water (polymer matrix refers to cuticles free of waxes) it was found that apparent surfactant solubility in waxes was only one-tenth of that in the polymer matrix.[24] If sorbed compounds were homogeneously distributed, the amount of surfactant in the limiting skin of the cuticles used in the present study was lower than those shown in Fig. 1B under UDOS conditions, i.e. under equilibrium between cuticle and aqueous solution.

In SOFU experiments, discrete amounts of radiolabelled solutes and surfactants are simultaneously applied to the outer surface of the CM. The water evaporates quickly leaving a solution of solute in neat hydrated surfactant. From this residue, surfactant homologues and radio-labelled solutes diffuse into and through the CM, each at its individual velocity (Fig. 10B). Eventually, they are collected in the receiver solution and are removed from the system when PLS is withdrawn during sampling. This process leads to decreasing amounts of both solutes and surfactants in the surface residue and to transient concentration gradients in the CM.

4.2 Verification of experimental procedures

The donor solutions contained different amounts of ethanol and therefore the area treated varied in UDOS experiments. However, this has no influence on the rate constants of desorption (k^*) if the diffusant is quantitatively sorbed in the cuticle during droplet drying. The rate constant of desorption is given by

$$k^* = \frac{P^* A}{V_{don}} \tag{4}$$

where P^* is a permeability (P) parameter independent of the partition coefficient K ($P = P^*K$) and A and V_{don} are the area and the underlying volume of the donor compartment of the cuticle, respectively.[17] The ratio of V_{don} and A corresponds to the thickness of the sorption compartment of the cuticle (Fig. 10) and in UDOS experiments this is constant. Thus, rate constants (k^*) should not be affected by the area covered by the donor droplet. This was experimentally confirmed (Table 3).

With phenylurea and cyanazine, PEG 400 was necessary to obtain rate constants of permeation by solubilising and improving sorption of these compounds into *Citrus* CM during evaporation of water of the donor solutions. If these compounds were applied in water only, rate constants were extremely low and could not be increased substantially even by very effective surfactants (data not shown). With *Pyrus* CM, sorption from aqueous donor solutions without PEG 400 was complete as indicated by desorption of up to 90% of the amount applied of these compounds with GP C-050 (Fig. 6). When PEG 400 was used on *Pyrus* CM no differences in the rate constants or surfactant effects were found, indicating that PEG 400 acted mainly on the sorption process, possibly by delaying crystallisation and prolonging the time for sorption into the inner surface of the CM. Furthermore, PEG 400 did not

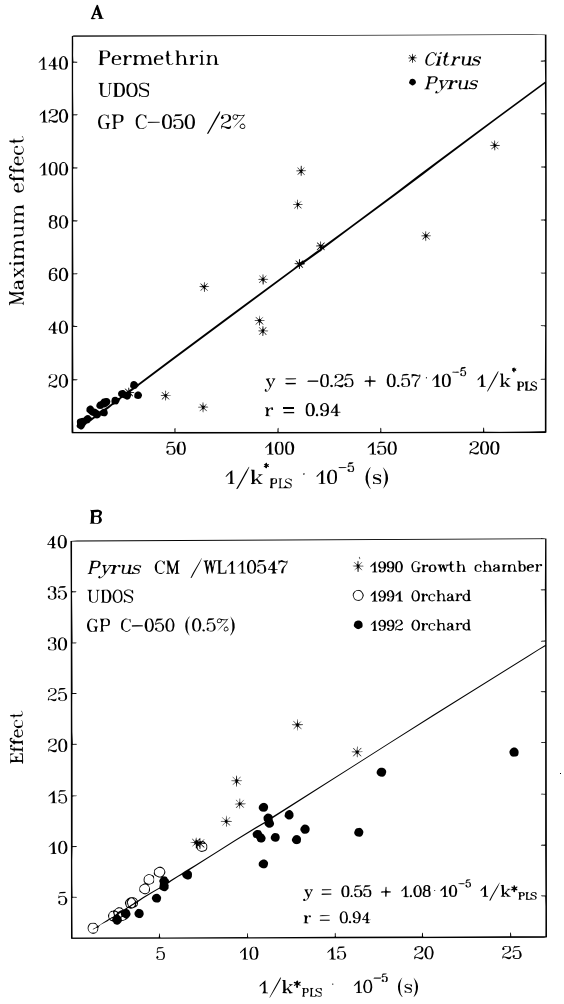

Fig. 11. (A) Dependence of the maximum effect of 'Genapol' C-050 on initial rate constants (k^*_{pls}) of permethrin in *Citrus* and *Pyrus* CM (data from Fig. 2B/D). (B) Dependence of the maximum effect of Genapol C-050 on initial rate constants (k^*_{pls}) of WL110547 in *Pyrus* CM of different origin.

change the permeabilities of the CM as shown by the lack of any effect on the mobility of phenylurea and cyanazine with *Pyrus* leaf CM (Figs 7A, 7C). Probably most of the compound was sorbed in the CM and not dissolved in PEG 400 on its inner surface, otherwise the rates would have been lower owing to an increase in the donor volume. It is theoretically possible that it acts in that way and simultaneously increases rate constants. Thus the two effects may cancel each other, thereby making it impossible to find any influence in the control experiments with phenylurea and cyanazine with *Pyrus* CM. However, PEG 400 contains more than 100% water under saturation humidity at the loading side and this may support our assumption that most of the compound was actually sorbed into the CM.

4.3 Effects of Genapol surfactants on mobilities of solutes in CM

The results from the UDOS experiments with *Citrus* and *Pyrus* CM show that the lower ethoxylated analogues of these alcohol ethoxylates greatly increased solute mobilities in these isolated CM (Figs 2 and 3). GP C-050 substantially increased mobilities of all compounds and its effect was larger than that of GP C-080 and GP C-100, while GP C-200 was nearly inert (Figs 3–5). Accelerating activity of ethoxylated alcohols is proportional to the amount sorbed in cuticles and waxes.[24–26] In UDOS experiments the numbers of carbon atoms and ethoxy groups influence the amount sorbed (eqn (3)) and hence their intrinsic accelerator activity. The results of this study also show that the amounts of surfactant sorbed in cuticles decreased in the same order as their general effects on solute mobility, i.e. GP C-050 > C-080 = C-100 > C-200 (Fig. 1B).

However, there is one small discrepancy in that practically the same amounts of GP C-080 and C-100 are calculated to be sorbed at equilibrium, while GP C-080 was clearly more effective as an accelerator than GP C-100 (Fig. 3). Possibly, the actual composition of the GP C-100 lot used differed from the analytical data provided by Hoechst, or equilibrium sorption was not obtained with all CM, such that actual surfactant concentrations in the cuticles were smaller than those calculated. Data generated using *Pyrus* CM (Fig. 5) which are more permeable than *Citrus* CM, indicate that equilibrium might not have been obtained with all CM. With the even larger GP C-200 this problem would be even greater. From the data by Riederer *et al.*[25] cuticle/water partition coefficients of surfactant homologues can be calculated and a value of 3·54 for a surfactant having the formula $C_{12}E_{23}$ is obtained. Thus, even the most polar homologue in our series was lipophilic and more soluble in cuticles than in water. Failure of GP C-200 to increase solute mobility in cuticles could be due to the too low volume fraction sorbed in equilibrium. Data in Fig. 1B indicate that a significant amount of surfactant (volume fraction >0·018) should be sorbed at equilibrium with GP C-200, yet this surfactant was nearly inert in this study (Figs 3–5). This is in accordance with the results obtained with monodisperse surfactants, since extrapolation of the effect/volume fraction relationship for monodisperse fatty alcohol ethoxylates to low volume fractions results in an effect of 1 (i.e. no increase at a volume fraction of approximately 0·03).[25]

4.4 Acceleration as affected by initial mobilities in cuticles

With all model compounds studied, the effect of GP C-050 varied with the initial rate ($1/k^*_{PLS}$) of *Pyrus* CM (Fig. 7) and also, though less pronounced, with *Citrus* CM (data not shown). Initial mobilities and $1/k^*$PLS values of the CM of CM populations used in the experiments differed considerably (cf. Fig. 7).

Effects of GP C-050 depended on initial mobilities in cuticles. The lower the initial mobility, the greater was the accelerator effect. This holds not only for a given species, but is valid also for a given compound and cuticles from different species (Fig. 11A) or from the same species coming from different years or locations (Fig. 11B). In Fig. 11A the effect of GP C-050 on mobility of permethrin is plotted against $1/k^*_{PLS}$ for both *Citrus* and *Pyrus* CM. The line drawn fits the combined data reasonably well ($r^2 = 0{\cdot}88$). A similar result was obtained for different *Pyrus* CM (cv. 'Bartlett') which were isolated from leaves of trees growing in a growth chamber (1990) or in an orchard (1991 and 1992), respectively. Effects of GP C-050 on mobility of WL110547 differed among samples, but a straight line could be fitted to the combined data (Fig. 11B).

4.5 Acceleration as affected by molar volume of solutes

In Fig. 12 mean rates of desorption (log k^*) with PLS (empty dots) for the model compounds (combined values from all experiments obtained with the respective compound and species) are plotted against molar volume (V_x). With both *Citrus* and *Pyrus* CM, rate constants of desorption depended exponentially on molar volume V_x of the model compounds, as indicated by the linearity of the plot of log k^* against V_x (empty dots in Figs 12A and B). At high volume fractions, chlorfenvinphos and (to a lesser degree) permethrin, both lipophilic liquids, act as self-accelerators. While the intrinsic mobility (volume fraction $\rightarrow 0$) for chlorfenvinphos in *Pyrus* CM was obtained recently,[26] such a value for permethrin is not yet known. This is the reason why the value for permethrin, which had to be applied in the highest amount (Section 2.3.1) with *Pyrus* CM (Fig. 12B), is larger than predicted from its size.

Accelerator surfactants increased mobilities of large solutes much more than those of smaller ones (Fig. 3). For both species the slope of the line of a plot log k^* versus molar volume decreased substantially if GP C-050 was used as desorption medium instead of inert PLS (filled dots in Figs 12A and B). In Fig. 12, log k^* values for desorption with GP C-050 (filled dots) with *Citrus* were calculated by multiplying initial rate constants by the mean effects for each compound (Table 4) while for *Pyrus* CM initial rate constants were multiplied by the more correct (Section 4.3) effects at the mean value of k^*_{PLS} (Fig. 7).

In this particular set of compounds, size (molar volume) and the lipophilicity (log K_{ow}) are correlated ($r^2 = 0{\cdot}83$) and, since there would also be a reasonable correlation for a plot of log k^* against log K_{ow}, plotting effects against molar volumes may appear arbitrary (Fig. 3). However, this possibility can be clearly excluded, since the existence of a correlation with lipophilicity (Table 2) would mean that the rate constants

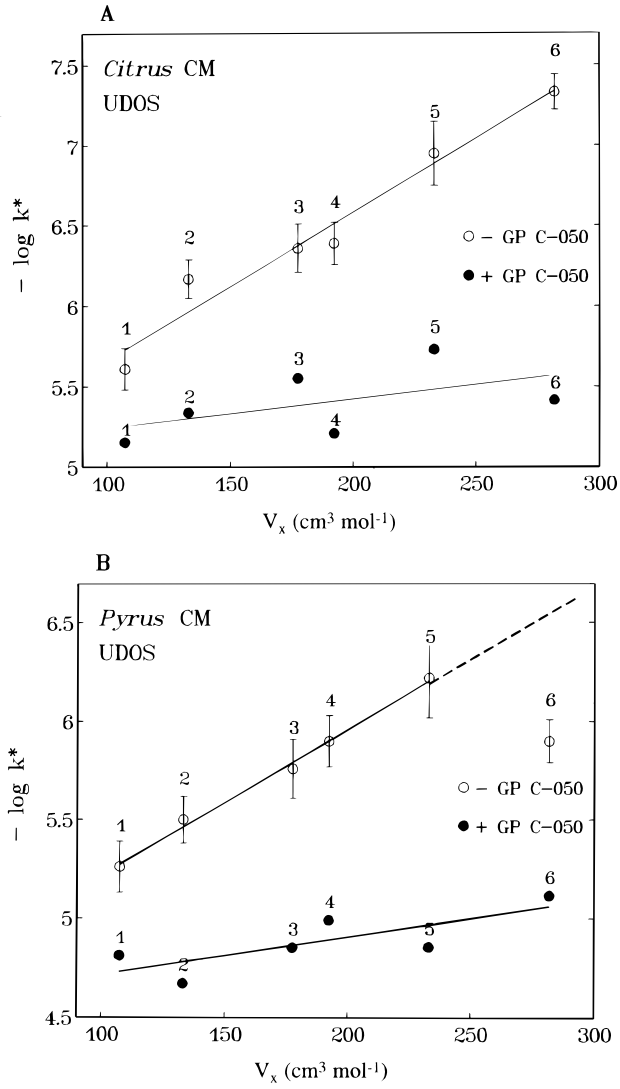

Fig. 12. Dependence of rate constants of desorption of the model compounds on their molar volumes (V_x) (○) without and (●) with 'Genapol' C-050 for (A) *Citrus* and (B) *Pyrus* cuticular membranes. Each point represents the mean of 17 to 89 CM. For compound identification see Table 2.

increase from phenylurea to permethrin, while the opposite was observed (Fig. 12). Rate constants as measured using UDOS are proportional to diffusion coefficients, which are generally independent of lipophilicity but decrease rapidly with the size of diffusants.[39] This has been shown for diffusion of a large number of compounds in CM, reconstituted cuticular waxes, human skin and synthetic polymers.[13,22,24,39–43] Due to high viscosity, the size selectivity of a solid matrix is much more pronounced than that in liquids.[40] Alcohol ethoxylates decrease the viscosity of cuticular waxes and increase their fluidity, i.e. they become more like a liquid, which results in a reduction of their size selectivity. This is the reason why mobilities of large molecules are more affected by accelerators such that effects increase with solute size and differences between compounds disappear (Fig. 12).

4.6 Effects of surfactants on rates of penetration

In SOFU experiments, rates of penetration (J, mol m^{-2} s^{-1}) are measured, which depend on the permeance (P) of cuticles and on the concentration difference across cuticles:[13]

$$J = P\,\Delta C \tag{5}$$

Permeance is a composite quantity which depends on solute mobility (D) in cuticles, a partition coefficient (K) as measure of differential solubility and the thickness of the CM (Δx_{CM}):

$$P = \frac{DK}{\Delta x_{CM}} \tag{6}$$

D is related to UDOS rate constants (k^*)

$$D = k^*\,\Delta x_{ls}\,\Delta x_{soco} \tag{7}$$

where Δx_{ls} and Δx_{soco} are the thickness of the limiting skin and the sorption compartment, respectively, as shown in Fig. 10. Combining eqns (6) and (7) and substituting into eqn (5) yields

$$J = \frac{k^*\,\Delta x_{ls}\,\Delta x_{soco}\,K}{\Delta x_{CM}}\,\Delta C \tag{8}$$

which can be further simplified since the thickness of the sorption compartment and the total thickness of the CM are similar:

$$J = k^*\,\Delta x_{ls}\,K\,\Delta C \tag{9}$$

Equation (9) shows that rates of penetration (J) are proportional to solute mobility (k^*) differential solubility (K) and the concentration difference across the cuticle. The thickness of the limiting skin is constant for each type of CM and needs no further consideration. If the donor compartment (surfactant residue) is constant, penetration can be treated as a desorption experiment and linear graphs obtained.

We have shown above that all surfactants, except GP C-200, significantly increase solute mobility k^* and, for a given type of CM and solute, this increase depends only on surfactant concentration in the CM. In UDOS, surfactant concentrations increase with time and eventually become constant, if surfactants penetrate into CM fast enough relative to the duration of the experiment. In SOFU experiments, surfactant concentrations also increase with time, but only with slowly penetrating surfactants do their concentrations become stationary within the cuticle, since only a finite amount of surfactant is applied. Fast-penetrating surfactants diffuse into the receiver and are removed from the system. Therefore, mobilities of solutes in the presence of accelerators are often not constant in SOFU, rather they increase initially and eventually decrease as the supply of surfactant in the surface residue and in the CM become depleted.

The term $K\,\Delta C$ can be considered as the driving force of penetration[13] and, with SOFU experiments, the term can be simplified because the solute concentration in the water of the receiver is held practically zero owing to the large volume of the receiver and the sorption of lipophilic solutes in PLS vesicles. Thus, the driving force depends only on the concentration in the surface residue, which serves as a donor once the bulk water has evaporated, and we can write the driving force as KC_{don}. The solute concentration in the surface residue changes with time, since both solutes and surfactants penetrate into cuticles. These changes cannot be quantified at present, since we only measured the permeation of the solutes and not the surfactants.

The partition coefficient (K) represents the equilibrium concentration of solutes between the limiting skin and the surface residue. This quantity is not known either, and it may not be constant, since composition of the surfactant residue will change with time because the smaller and more lipophilic homologues are likely to penetrate faster and leave the larger and more polar homologues behind.

Owing to these complexities and unknown variables, we can discuss SOFU results only qualitatively. Rates of penetration of WL110547 followed approximately first-order kinetics, both in the absence and presence of GP C-200. That is, the concentration of this lipophilic solute in the surfactant residue on top of the CM decreased exponentially with time. Rate constants (k) were higher in the presence of GP C-200 than in the control and increased with increasing amount of surfactant up to 50 μg (Fig. 8B). Observed rate constants approximately doubled on increasing GP C-200 from 5 μg to 50–100 μg. GP C-200 may increase penetration by dissolving solid WL110547 on the cuticle surface, thereby increasing the driving force, but the increase of rates with concentration indicated that some homologues may also have penetrated into the limiting skin and acted as plasticisers while most remained on the surface. The effect of this surfactant in this SOFU experiment is greater than that in UDOS (Figs 5D and 6D) because penetration from the neat surfactant residue is faster than from the micellar solution and depends only on solubility in the cuticle and not on the relative solubility, i.e. the partition coefficient. The fact that acceleration did not increase with increase in application of GP C-200 from 50 to 100 μg is most probably due to dilution of the solute in the surfactant, or in terms of eqn (9), the decrease in C_{don} on doubling the amount of surfactant counteracted the surfactant effect on mobility (k^*). We have shown before that, in the

presence of surfactants which do not penetrate cuticles (such as 'Tween' 80), rates of uptake decrease with increasing amounts of surfactant.[13]

GP C-050 increased the rates of penetration of WL110547 much more than did GP C-200 though the rates of penetration were not first-order throughout the period of penetration with the highest rates in the 0 to 6-h interval (Fig. 8A). This relatively smaller and more lipophilic surfactant penetrates cuticles rapidly and therefore achieves maximum concentrations in the limiting skin and maximum effects on solute mobility quickly. Surfactant effects increased with increasing amounts of surfactant, though it can be seen that the effect of adding 100 μg of GP C-050 initially produces a slower rate than 50 μg. This may be accounted for by the competition between sufficient amounts of GP C-050 for modifying the cuticle, thus accelerating WL110547 penetration, and excess amounts acting to dilute WL110547 in the reservoir, thus reducing concentration and the driving force. At later intervals the larger reservoir of 100 μg GP C-050 will eventually reduce, due to penetration, thereby increasing the driving force and eventual penetration of WL110547.

Similar results were obtained with the smaller and more polar solute, cyanazine (Fig. 9). GP C-050 greatly increased rate constants (maximum $1{\cdot}4 \times 10^{-5}$ s^{-1}) though the dilution effect with 100 μg prevailed throughout the experiment, over 25 h. As with WL110547, GP C-200 increased the rate constants of cyanazine penetration, but the effects of different amounts of the surfactant were small and not significant.

So far the effect of the partition coefficient (K) has not been considered. Equation (9) suggests that, at constant driving force, rates of penetration should be higher for lipophilic than for polar solutes. Rate constants of penetration in the absence of surfactants are not very different with values of $2{\cdot}2(\pm 0{\cdot}7) \times 10^{-6}$ s^{-1} for cyanazine and $1{\cdot}5(\pm 0{\cdot}75) \times 10^{-6}$ s^{-1} for WL110547 nor are mobilities (k*) with values of $1{\cdot}75 \times 10^{-6}$ s^{-1} (cyanazine) and $1{\cdot}26 \times 10^{-6}$ s^{-1} (WL110547). K_{ow} values for cyanazine and WL110547 are 126 and 3982, respectively, which implies that WL110547 is about 30 times more lipophilic than cyanazine (Table 2) and penetration should be better with this compound. However, in SOFU experiments, solutes are not dissolved in water but in neat hydrated surfactant, and the cuticle/surfactant partition coefficients are not known. Owing to the good solvent power of surfactants and many other adjuvants, especially for lipophilic solutes,[35] these partition coefficients are much smaller, and differences between compounds may also be reduced. For instance, chlorfenvinphos has a cuticle/water partition coefficient of more than 1000, while the partition coefficients between cuticle and PEG 400 or GP C-050 are smaller than 1, i.e. chlorfenvinphos is more soluble in these adjuvants.[27]

No surface residue of GP C-050 was visible at the lower amounts applied (5 and 10 μg) after the third sample (20 h) and therefore both the effect on solute mobility and on the physical state (dissolution) of the compounds in the surfactant change. A comparison of rates of penetration between cyanazine and WL110547 in the presence of surfactant must therefore be limited to the higher amounts of surfactants and to the time period up to 6 h. The values for cyanazine and WL110547 differ by a factor of 2·5 (1·4 and $3{\cdot}5 \times 10^{-6}$ s^{-1}). Since the applied concentrations did not differ and UDOS experiments showed that in the presence of GP C-050 mobilities of different compounds are similar (Fig. 12), partition coefficients are expected to be quite similar.

4.7 Comparing these results to previous studies on foliar uptake

We have shown that 'Genapol' surfactants affect both solute mobilities and driving forces. 'Genapol' C-050 was sorbed in CM and penetrated cuticles most rapidly. It had the highest effects on solute mobilities for all solutes in the current range, with effects increasing with increasing size of the solutes. Increasing degrees of ethoxylation of the surfactants reduced sorption from aqueous micellar solution into cuticles and their effects on solute mobilities.

Stock *et al.*[9] measured uptake into the intact leaves of different plant species, while, in this study, isolated astomatous cuticles were used. In fact, we compared the rates of penetration of solutes and cuticular transpiration of isolated and non-isolated cuticles and we found slight differences (Baur[35] and unpublished results). However, for cuticular transpiration the coefficients of variation are large, above 30%, for studies both with isolated cuticles and where intact leaves of many different plants are used, and the range of values overlap[45,46] and coefficients of variation are even higher for organic solute mobility. Permeabilities of isolated CM tend to decrease somewhat during storage, possibly due to loss of volatile constituents which will modify the cuticle[21] and/or rearrangement of the cuticular waxes.[29] Such changes can affect absolute rates, though there is no reason to expect that the differential effects of molar volumes and lipophilicities of solutes and the effects of surfactants on penetration would be affected. The beneficial use of isolated cuticles for mechanistic studies has been discussed and underlined repeatedly.[47–49] The main drawback against direct comparison of the results in this work to the results of studying the patterns of uptake into living leaves is that enzymatically isolated cuticles can only be obtained from a limited number of species that do not have amphistomatic leaves.

This work was undertaken with the intention of obtaining a better understanding of the mechanisms of

adjuvant action underlying the observed pattern that penetration of lipophilic compounds was better assisted by surfactants with low ethylene oxide contents and that of hydrophilic compounds by surfactants with high ethylene oxide content.[8] The same range of compounds was used, though, in this paper, the results are given only for the lipophilic compounds and a chemically similar, though not identical, range of surfactants. The subsequent discussion will be limited to the action with these lipophilic compounds, i.e. with log K_{ow} values >0. Both studies with leaves and isolated cuticles have shown that fatty alcohol surfactants can penetrate cuticles, and those with low E content penetrate faster than those with high.[12,20] It can be seen that, in the context of this study, surfactants in deposits on leaf surfaces can have two major functions. Firstly, they can dissolve the penetrant, and that dissolution affects its concentration and partition coefficient and therefore its driving force for permeation. Secondly, after penetration into the cuticle they can, by a plasticisation mechanism, reduce the resistance to diffusion in the regions in the cuticle through which the penetrant will diffuse.[26] A possible reduction in the degree of crystallinity of waxes has not yet been shown. In deposits on leaf surfaces (or isolated cuticles in the SOFU experiments of this work) both functions are kinetic in the sense that, since the surfactants will begin penetrating into the cuticle during and after evaporation of the carrier solvents, both the driving forces and the cuticular resistances are being modified. These modifications will vary throughout the whole sequence of events and will be dependent on the structure of both the surfactant (ethylene oxide content in this work) and the penetrant.

The results of this work have reinforced these notions. From the UDOS experiments it is clear that the lowest ethoxylated surfactant, GP C-050, was the most effective at reducing the resistance of the CM and inducing accelerator action, and that accelerating action across the *Citrus* cuticular membrane (Fig. 3) was greatest for the largest compound, permethrin, and least for the smallest compound, phenylurea. In a similar study with *Pyrus* CM and WL110547 and cyanazine, a similar relationship was observed. For the hydrophilic surfactant with the most ethylene oxide units, GP C-200, there was little or no accelerating action and it can be concluded that there was little or no partitioning of it into the CM, at least from the aqueous solutions employed in the membrane cells.

In the SOFU studies with the more permeable *Pyrus* CMs, it was again shown that GP C-050 was more effective than GP C-200 with both a larger, lipophilic compound, WL110547, and a smaller, less lipophilic compound, cyanazine. Interestingly, in these SOFU studies, although GP C-200 was less effective than GP C-050, it did have significant accelerating action for both WL110547 and cyanazine which it did not in the UDOS studies. GP C-200 improved penetration in contrast to PEG 400 and this suggests that it is taken up in significant amounts from neat surfactant residues inducing some accelerator effect. This is indicated also by the fact that the compositions of GP C-050 and GP C-200 have more than 6% identical constituents (Fig. 1A). These results are in good accord with those observed by Stock *et al.*[9] in their work on leaves attached to plants and reinforce the conclusion that the penetration of lipophilic, larger compounds is best aided by surfactants with low ethylene oxide contents. The phenomenon is probably best rationalised in terms of molar volume, since rates of diffusion are inversely related to a molar volume term and any quantification in terms of partitioning or solubility will, in any case, be difficult owing to the transient, varying nature of the cuticular wax/surfactant composition during the penetration process. Furthermore, lipophilic solutes will have higher permeabilities since their solubilities in the cuticle are likely to be higher providing crystallisation/solidification (and hence reduction effects on the driving force) is avoided.

Further mechanistic aspects of surfactants' mode of action in foliar uptake and studies on the mechanisms for penetration of hydrophilic compounds will be the subject of a forthcoming paper.

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